VALIDATION OF A LABORATORY DEVELOPED PCR TEST FOR USE IN THE DIAGNOSIS OF HIV-2

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2019 CDC HIV Diagnostics Conference

BACKGROUND

The CDC HIV Diagnostic algorithm recommends use of HIV assays that detect and differentiate HIV-1 from HIV-2; the Bio-Rad GeeniusTM HIV 1/2 Supplemental Assay is the only U.S. FDA approved supplemental confirmatory assay with such capability. Bio-Rad Geenius HIV 1/2 indeterminate or negative specimens require resolution by HIV-1 or HIV-2 nucleic acid testing (NAT); however no U.S. FDA approved HIV-2 NAT assays are available.

Due to low plasma viremia in many HIV-2 infected individuals, we developed a qualitative Total Nucleic Acid, reverse transcription PCR (HIV-2 TNA) assay for detection of HIV-2 RNA/DNA in cell pellets. The assay was validated for clinical specificity/sensitivity, subtype detection sensitivity, accuracy, lower limit of detection (LLOD) and interference for use in the classification of HIV-2 infection status.

MATERIAL AND METHODS

HIV-2 Total Nucleic Acid (TNA) Laboratory Developed Test (LDT): The HIV-2 TNA assay targets the HIV-2 5' long terminal repeat (LTR/R/U5) region. HIV-2 RNA/DNA was extracted using Qiagen QIAamp Blood Mini kit (Valencia, CA) from cell pellets (0.5 ml of EDTA blood) after lysis of erythrocytes (See controls). Extraction and amplification efficiencies were monitored using the Applied Biosystems® Human GAPD (GAPDH) endogenous control kit. Amplification was performed on the 7500 Fast Dx Real-Time PCR instrument.

Controls and Lower Limit of Detection (LOD) Panels: Positive controls at 100 HIV-2 cultured cells per ml blood and LLOD panels were prepared by spiking HIV-2 HUT78/HIV-2 D194 (NIH AIDS Reagent Program Cat# 119) cells into EDTA whole blood obtained from HIV negative donors (Biological Specialty Corporation). Erythrocytes in 0.5 ml EDTA blood were lysed using Red Blood Cell Lysis buffer (Roche Cat No. 11814389001) and cells were pelleted by centrifugation at 13,200 x g for 3 minutes. Cell pellets were stored at <-70°C until tested. Negative controls were prepared from EDTA blood in a similar manner.

Analyte Specificity, Sensitivity, and Interference Samples: HIV-1, HIV-2, HBV and HCV viral stocks were spiked at 10 ul of 1E6 to 1E7 copies/mL into cell pellets from HIV negative donors to generate a panel of samples to assess the specificity and sensitivity of the HIV-2 TNA PCR LDT.

Clinical Samples: Twenty-five residual, post-test viral load HIV-2 samples were received from Dr. Linda Styer, Wadsworth Center, NYS Department of Health under a Material Transfer Agreement. Specimens were provided anonymized (blinded), with no personal identifiers. HIV-2 positive whole blood was purchased from CepheusBio.

Analysis: Sensitivity and specificity calculations were performed using MedCalc (https://www.medcalc.org/calc/ diagnostic_ test.php). Prism 7 (GraphPad Software, La Jolla, CA), Excel and EP Evaluator LLC (Data Innovations, Burlington, VT) software packages were used for LLOD and Clinical Specificity and Sensitivity.

Regulatory Approval: Clinical samples were tested under a Non-human Subject Research study approved by Walter Reed Army Institute of Research (WRAIR) Human Subject Research Protection Branch.

RESULTS

	Negative Reference	Positive Reference	Total
Negative HIV-2 TNA	51	3	54
Positive HIV-2 TNA	--	21	21
Total	51	24	75

Table 1: Clinical Specificity and Sensitivity. The HIV-2 TNA test demonstrated 100 % specificity and 88-92% sensitivity compared to the Wadsworth Qualitative and Quantitative NAT tests respectively.

Sample ID	Subtype	GAPDH Ct	HIV-2 Ct
HIV-2 CBL-23	A	23.02	21.44
HIV-2 CBL-20	A	23.18	23.3
HIV-2 MVP-15132	A	23.5	22.15
HIV2 D194	A	23.75	23.43
HIV2 7924A	A	23.97	28.33
HIV2 60415K	A	22.55	28.65
HIV2 CDC 310248	A	23.86	31.36
HIV2 CDC 310072	A	23.56	31.28
HIV2 CDC 77618	A	23	28.76
HIV2 CDC 310319	B	23.15	30.03
HIV2 PB001290206	B	22.59	30.49

Sample ID	Subtype	GAPDH Ct	HIV-2 Ct
HIV-1	A	23.40	TND
HIV-1	A/G	23.37	TND
HIV-1	A/E	22.45	TND
HIV-1	B	22.10	TND
HIV-1	D	22.98	TND
HIV-1	C	23.01	TND
HCV	--	24.13	TND
HBV	--	24.09	TND
HIV-2 + HCV	--	23.73	24.24
HIV-2 + HBV	--	24.14	26.02

Table 2: Subtype Detection, Sensitivity and Interference. All contrived samples made using HIV-2 subtypes Group A (9) and Group B (2) were detected. Contrived samples to test interference showed the assay to be highly specific for HIV-2 only with no cross-reactivity to HIV-1, HBV or HCV.

	Negative Control GAPDH	Positive Control GAPDH	Positive Control HIV-2	Negative Clinical GAPDH	Positive Clinical GAPDH
N	22	42	42	55	23
Mean Ct	24.23	24.00	26.14	23.21	23.35
SD	0.73	0.82	2.23	1.41	1.59

Table 3: Precision. The HIV-2 positive control (50 HIV-2 D194 cells/pellet) mean value cycle threshold (Ct) was 26.1 with a SD of 2.2 Ct; GAPDH control mean was 24.1 Ct with a SD of 0.8 Ct. The GAPDH endogenous control mean within clinical samples was 23.26 Ct with a SD of 1.5.

Cells/mL	0.1	1	12.5	50	100
% Detected	20%	50%	85%	100%	100%
N	10	10	40	40	40

Table 4: Lower Limit of Detection (LLOD). Assay LLOD at 95 % detection was 34 cells/ml and 50 % at one HIV-2 cell/ml.

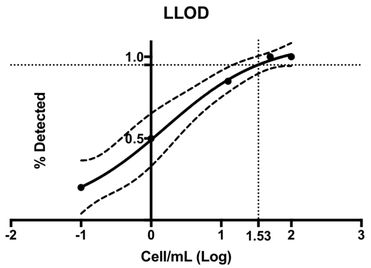

Figure 1: Graphical Analysis of LOD data. Reflects a 34 cell/ml (1.53 $\log_{10}$ cell/ml) LLOD for 95% detection.

The confidence level based on 40 positive replicates, with a type II risk (β) set at 0.05, is 92.70% that the LLOD of the assay is at or below 50 HIV-2 cells/mL.

The confidence level based on 80 positive replicates, with a type II risk (β) set at 0.05, is 96.30% that the LLOD of the assay is at or below 100 HIV-2 cells/mL.

CONCLUSIONS

- The HIV-2 TNA test is highly specific for HIV-2 RNA/DNA detection in whole blood.
- The lower assay sensitivity for clinical specimens may be due to use of residual, post-test, viral load HIV-2 specimens that had experienced repeated freeze-thaws.
- Overall the test demonstrated modest sensitivity, high precision, and high specificity.
- The assay continues to be developed and evaluated as a supplemental test for confirmation of HIV-2 infection.

Disclaimer and Funding

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation. This work is supported in part by the US Army Medical Research and Materiel Command under Contract No.W81-XWH-18-C-0337 and W81-XWH-16-C-0225 and by the US Military HIV Research Program, the Walter Reed Army Institute of Research under a cooperative agreement (W81-XWH-11-2-0174) with the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.